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No. 3 JANUARY 17th, 1959 Vol. 71

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The Effects of Chlortetracycline on the Development of *Eimeria tenella* in Chicks

BY

L. P. JOYNER

Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Weybridge, Surrey

SUMMARY.—1. The coccidiostatic effects of chlortetracycline have been investigated and it was found that 0.08 per cent. of the antibiotic in the food gave complete control of the mortality due to Eimeria tenella *provided that administration commenced at the time of infection.*

2. 0.08 per cent. of chlortetracycline in the drinking water did not give such complete protection and there was again a marked reduction in efficacy when administration was delayed.

3. The main effect of the antibiotic appeared to be a retardation of schizogony. Abnormal segmentation of the second generation schizonts was observed histologically.

ALTHOUGH the main use in poultry husbandry has been as a growth stimulant, it has been known for some time that chlortetracycline* possesses coccidiostatic properties. Seeger, Lucas and Tomhave (1950) first reported the control of *Eimeria tenella* using 900 g. of chlortetracycline per ton (approximately 0.09 per cent.). Cuckler and Malanga (1956) confirmed this observation using 0.025 per cent. and 0.1 per cent. in the food. Marthedal and Velling (1953) also observed some coccidiostatic effect when the antibiotic was given in the drinking water at the relatively low concentration of 50 mg. per litre.

The reports by Blount (1954), White-Stevens, Zeibel and Smith (1955) and more recently, the investigations of Gardiner (1957) have shown that advantages are to be gained by using chlortetracycline in conjunction with established coccidiostats. By this means the retardation of growth resulting from drug toxicity or sublethal coccidial infections could largely be overcome.

Investigations in this laboratory on the effects of chlortetracycline on coccidia-infected chickens were begun by examining in some detail the coccidiostatic properties of the antibiotic. The experiments reported in this paper were carried out with this object.

Materials and Methods

Rhode Island Red or White Leghorn chicks aged 2 to 4 weeks were maintained in wire floored heated hovers and given unrestricted food and drink. Chlortetracycline was administered in the food as a commercially available premix containing 10 gm. chlortetracycline per pound, which was incorporated in the standard chick mash with the aid of a mechanical mixer. For administration in the drinking water, a commercial soluble product containing 25 gm. chlortetracycline per lb. was used. The concentrations used in the various experiments were within the ranges of 0.02 to 0.08 per cent. (200 to 800 gm. per ton approx.) in the food and 0.01 to 0.08 per cent. in the drinking water.

Chicks were infected by the oral administration of sufficient *E. tenella* oocysts to kill the majority of untreated birds. The caeca were examined *post-mortem* to make sure that the lesions were consistent with death from caecal coccidiosis.

Histological preparations were obtained from caecal tissue removed from chicks killed after varying periods following infection with 1,000,000 oocysts. The caeca were removed, opened longitudinally, lightly washed in saline and rolled round a small

* Aureomycin.

tube of stiff paper before immersing in Bouin's fluid. After about one hour, the tube of paper was removed and the coil of tissue returned to the fixative. The tissue was embedded in paraffin wax in the usual way and a section cut through the coil of tissue gave a longitudinal section of the caecal wall from one end to the other. The sections were stained with Erhlich's haematoxylin and eosin. In identifying the different stages of *E. tenella* particular care was taken to examine the whole length of the sections.

The Effects of Varying Dosage and Time of Administration

In the experiments recorded in Table I various concentrations of chlortetracycline were administered either in the drinking water or in the food. Some groups received the drug from the time of infection and others received it 48 hours later. In all groups the drug was removed on the fifth day after infection.

TABLE I

THE COCCIDIOSTATIC EFFECTS OF DIFFERENT DOSES OF CHLORTETRACYCLINE ADMINISTERED IN THE FOOD OR DRINKING WATER

Dose		Number of Deaths								Number of chicks per group
		In Food—per cent.			In Water—per cent.				Nil	
		0·08	0·04	0·02	0·08	0·04	0·02	0·01		
Experiment number	Start of treatment : –days after infection									
1	0	0	6	9					14	21
	0+2	8	12	6						
2	0	0	2	10		3	8	12	12	15
	0+2	7	8	8		3	14	12		
3	0	0	1	3	2	5	10		13	15
	0+2	4	5	13	9	13	13			

Treatment commenced at times stated and ceased 5 days after infection.

It will be seen that when 0.08 per cent. of chlortetracycline was given in the food, complete control of the disease could be obtained provided that administration began at the time of infection. Delay for 48 hours markedly reduced the efficacy of the treatment.

When the antibiotic was given in the drinking water, none of the doses used gave complete control, although mortality was again lower when treatment began at the time of infection.

To investigate whether the addition of chlortetracycline to the drinking water reduced the fluid consumption of the birds, an experiment was carried out with two groups of 25 chicks aged 17 days. The control group of normal birds drank 3,560 ml. of water in 3 days. The other group which received water containing 0.08 per cent. chlortetracycline, drank 3,171 ml. during the same period. This was a reduction of approximately 11 per cent., which probably would not account for a marked reduction in efficacy of the contained antibiotic under the conditions of these experiments.

The Susceptibility of Different Stages of *E. tenella* to Treatment with Chlortetracycline

Early administration appeared to increase the control of the infection and it was therefore of interest to determine which stages in the life cycle of *E. tenella* were the most susceptible to the action of the antibiotic. A series of experiments was therefore designed in which the antibiotic was given in the food at different times during the infection.

It will be seen from Fig. 1 (experiment A) that 48-hour courses of treatment with 800 gm. per ton of food (0.08 per cent. approx.) were too short to have a marked inhibitory effect upon the infection. When the period of treatment was extended to 3 days (experiment B), complete control was still not obtained, although the number of deaths was markedly reduced when the antibiotic was given from 2 to 5 days after infection. When therapy was delayed until 3 days after infection however, very little control was obtained. A similar result was obtained in the experiment (C) in which the antibiotic was given at a concentration of 1,600 gm. per ton. Almost complete control was obtained when administration began either on the first or the second day after infection.

When this high dose of chlortetracycline was given from the time of infection, there was a tendency for mortality to be delayed. It will be seen that an

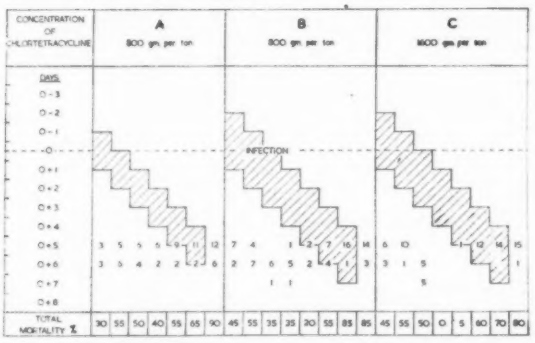

FIG. 1.—The effect of treatment with different doses of chlortetracycline in the food, for different periods of time on the development of *Eimeria tenella*. The shaded areas represent the periods of treatment. The figures indicate the incidence of deaths from caecal coccidiosis.

appreciable number of deaths occurred on the 7th day whereas in other groups the peak of mortality occurred on the fifth day. Such a delay would be consistent with a retardation of the development of the first generation schizonts which are present up to the 72nd hour after infection.

It was also noticeable that mortality was slightly reduced in several groups receiving the antibiotic early in the infection.

A further experiment was designed to observe was to retard the development of both the first and the second generation schizonts. This was also reflected in the appearance of the second generation schizonts themselves. Under treatment with the antibiotic many of the schizonts examined 120 hours after infection failed to segment completely and consisted of large residual masses with merozoites ranged radially round them as shown in Fig. 2. Normal schizonts at this stage divide completely into a large mass of merozoites arranged at random.

TABLE II

THE INCIDENCE OF STAGES OF *E. tenella* UNDER TREATMENT WITH 800 GM. PER TON CHLORTETRACYCLINE (0·08 PER CENT.)

Time after infection	Treatment	Stages of Development							
		Tropho-zoites	1st Gen. Schizonts		2nd Gen. Schizonts			Game-tocytes	Haemorrhage
			Early	Mature	Early Segmen-tation	Advanced Segmen-tation	Mero-zoites Formed		
48 hours	Chlortetracycline	++	++	+					
	Control	+	++	++					
72 hours	Chlortetracycline		++	++	+				
	Control			+	+++	+			
96 hours	Chlortetracycline				+++	++			
	Control				+	+++	+		+
120 hours	Chlortetracycline				++	+++			
	Control					+	+++	+	+
7 days	Chlortetracycline					++	++	+	+ (mucosa severely damaged)
	Control						all chicks dead		

The symbols " + " indicate the relative incidence of the different developmental stages.

histologically the effect of the antibiotic on the different stages of the parasite. Table II records the relative incidence of the easily recognisable stages in the development of *E. tenella* with and without treatment with chlortetracycline (800 gm. per ton). Two groups of chicks were infected with 1 million oocysts of *E. tenella*; one group received the antibiotic and the other served as a control. Two chicks from each group were killed at intervals after infection, the sections obtained from the caeca were examined and the relative incidence of the different stages was assessed as shown. This was at best a very rough measure of the rate of development of the parasite, but its greatest accuracy lies in the indication of the first appearance of certain stages. Thus, 72 hours after infection, there were many second generation schizonts in which segmentation had begun, whereas under treatment with the antibiotic, the majority of schizonts were still at the first generation stage. Similarly, mature schizonts and gametocytes were formed more rapidly in the absence of treatment. Haemorrhage also occurred more markedly and rapidly in the untreated infections. It is evident that the principal effect of the antibiotic

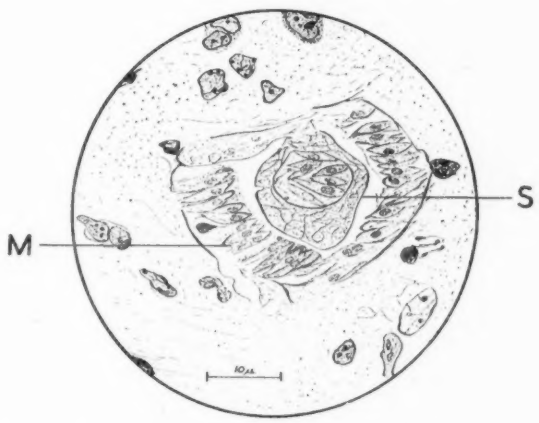

FIG. 2.—Schizont after 120 hours treatment with Chlortetracycline (800 gm. per ton). Showing central incompletely differentiated mass (S), with peripherally arranged merazoites (M).

Discussion

When chlortetracycline is administered to chicks infected with *E. tenella*, its growth-promoting effects, which are now generally recognised, may enable the birds to overcome the effects of sublethal infections more readily. Similarly the growth-retarding effects of slightly toxic coccidiostatic drugs may be reduced. A second beneficial effect would result from the direct action which the antibiotic has upon the parasite itself. Gardiner (1957a), using doses up to 200 gm. per ton, showed that "The addition of chlortetracycline to the diet benefited the chicks by stimulating their metabolism rather than by exercising any marked inhibitory effect on the coccidial organism," although a more recent report by this author suggests that the chlortetracycline may have some coccidiostatic effects (Gardiner, 1957b).

The experiments recorded in this paper show that chlortetracycline has marked coccidiostatic properties and provided sufficiently high doses are given, almost complete control of *E. tenella* can be obtained. The dose of 800 gm. per ton of food (approximately 0.08 per cent.) found to be necessary, agrees with the observations of Seeger, Lucas and Tomhave (1950) and Cuckler and Malanga (1956) who obtained good protection against *E. tenella* with levels of 900 gm. per ton and 0.025 to 0.1 per cent., respectively.

The present series of experiments has shown that although the antibiotic markedly suppresses the development of the second generation schizonts, it is necessary to commence administration at the time of infection in order to obtain the maximum effect. It has proved difficult to determine the reason for this necessity.

A likely explanation is that absorption and excretion of the antibiotic may be slow. Early administration would be necessary to obtain maximum blood or tissue levels and sufficient antibiotic may remain in the circulation after withdrawal of the antibiotic from the food, to cause slight inhibition of the parasite. This is supported by the observations recorded by Williams-Smith (1954) who found that chlortetracycline was very poorly absorbed after intramuscular or oral administration and very slowly excreted; detectable amounts being usually present in the serum 24 hours after a single dose. Slow excretion may be the reason why slight coccidiostatic activity apparently persisted after early withdrawal of the drug as demonstrated in Fig. 1.

Another possible reason why chlortetracycline apparently acts slowly, may be that it is not directly coccidiostatic, but by modifying the bacterial flora of the intestine, the parasite may be indirectly denied some essential growth substance. Similar effects by bacteriostatic substances including chlortetracycline against *Entamoeba histolytica* have been discussed by Neal (1958). It is probable that with this mode of action a certain time would be necessary before equilibrium with the intestinal micro-organisms could be reached. Similarly, once a steady state had been established, there would be some delay after withdrawal of the antibiotic before normal metabolism could be resumed.

It is evident that to obtain a worthwhile control of *E. tenella*, high doses of chlortetracycline are necessary and it is doubtful whether control by this means alone would be economic. Nevertheless, the present experiments have confirmed that chlortetracycline does possess coccidiostatic properties which might operate synergically with established coccidiostatic drugs to permit the use of reduced doses and at the same time obtain the benefits of growth stimulation by the antibiotic.

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PRESS ARTICLES AND BROADCASTING

From time to time members of the profession are invited to write, or they offer on their own account, articles for the press or material for broadcasting. B.V.A. headquarters would always be very glad to offer assistance, especially over matters of policy and the general presentation of work for lay readers or listeners. It is of great importance that public statements by veterinary surgeons are so framed as to give to the public a fully adequate idea of the work done by our profession, and of its standing.

INTERESTING BROADCAST PROGRAMMES

In the coming week there are several programmes which may be of interest to our readers, as follows: Sunday at 2 p.m., the weekly television farming feature which includes an investigation into methods of producing beef from dairy herds, and a consideration of systems of rearing beef calves; Thursday, Scottish Region (sound), "Farm Forum" at 7.30 p.m.; Saturday, Midland Region (sound), "Farming Magazine," 11.35 a.m.; and Saturday, Third Programme, "A Psychoanalyst Learns from Animals," 6.45 p.m.

THE WELLCOME FOUNDATION

Dr. D. G. ff. Edward, M.R.C.S., L.R.C.P., head of the virus department of the Wellcome Foundation, has been invited by the New York Academy of Sciences to be chairman of a "Conference on the Biology of Pleuropneumonia-like Organisms," which the Academy arranged at the Hotel Barbizon-Plaza in New York on January 14th, 15th, and 16th. Dr. S. E. Piercy, D.SC., F.R.C.V.S., also of the virus department of the Wellcome Foundation, has been invited to give a paper before the conference.

A New Method of Preparing Smears of Bovine Faeces for Microscopical Examination in the Diagnosis of Johne's Disease

BY

M. P. CUNNINGHAM and N. J. L. GILMOUR

University of Glasgow Veterinary School

SEVERAL methods have been used in the past in preparing bovine faeces for the detection of *Mycobacterium johnei* microscopically, namely: direct smear (Twort & Ingram, 1913; Cauchemez & Sergent, 1934; Davies, 1945), centrifugation (Sheather, 1932), concentration using antiformin (Minett, 1933), and flotation methods (Smith, 1938; Smythe, 1950).

Comparisons of the above methods led most authors to the conclusion that the direct smear technique gave the best results (Cauchemez & Sergent, 1934; Levi, 1948; Doyle & Spears, 1951; Soltys, 1951).

In this paper we describe a method of preparing faeces for microscopical examination and compare it with the methods most commonly used.

Technique

1. Place sufficient faeces on the centre of a slide to cover 2 square centimetres of approximately 0.25 cm. thick.
2. If necessary add water to make the faeces of the consistency of thick gruel. Mix slightly without spreading and leave for 5 minutes.
3. Take another slide and place its long edge in contact with and at right angles to the surface of slide no. 1, adjacent to but not quite touching the faeces (Fig. 1).
4. Using a glass rod thoroughly mix the faeces and spread it along the junction of the 2 slides (Fig. 2).
5. Lift slide no. 2 cleanly, leaving a straight edge of faecal stained fluid.
6. The smear is now dried slowly. An electric hot plate at 50° to 70° C. for 5 to 10 minutes gives good results, but the slides may be dried at room temperature, in an oven, or on a biscuit tin lid over a bunsen.
7. When the slide is thoroughly dry remove from hot plate and allow to cool.
8. When cool, remove excess faeces with a sharp scalpel taking care not to interrupt the continuity of the straight edge. The preparation is then dusted with a piece of gauze.
9. Stain by the Ziehl-Neelsen method and decolorise for 30 minutes in acid alcohol.

Figure 3 illustrates the result.

There is a zone along the edge of the smear (double dotted line on Fig. 3) where, on evaporation of the water, fine particles of faecal debris have been deposited. It is in this zone that most organisms are found.

Comparative Trial

Methods for comparison

1. New method as described above.
2. Direct smear as described by Davies.
3. Centrifugation as described by Sheather.

The smears were stained by the Ziehl-Neelsen method and decolorised in acid alcohol for 30 minutes.

Trial A. Seventeen samples of naturally infected bovine faeces in which single acid-fast bacilli predominated were used. As these samples were received for routine diagnostic examination the clinical status of the animals from which the samples were taken is unknown.

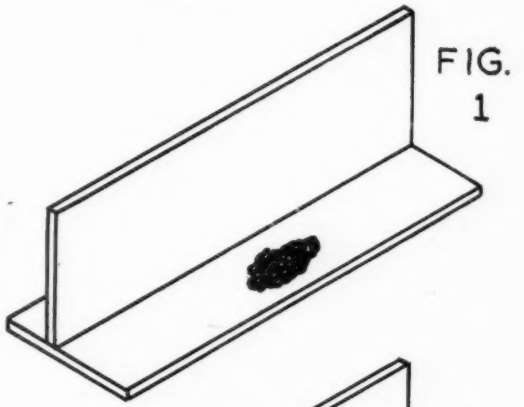

FIG. 1

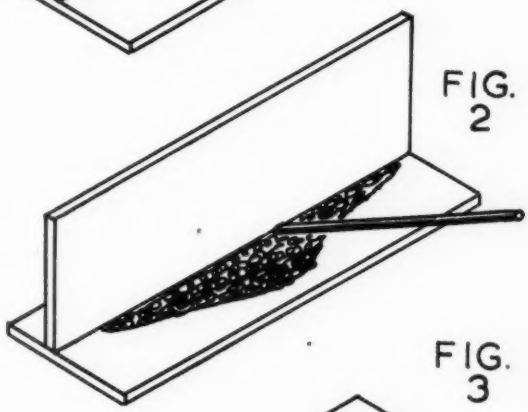

FIG. 2

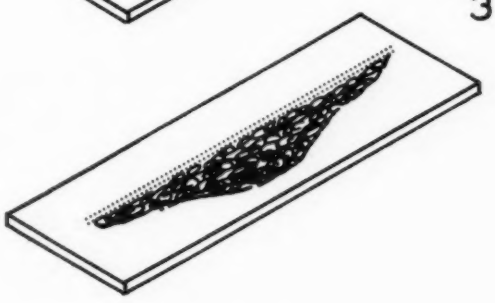

FIG. 3

Each smear was examined for 5 minutes and the number of acid-fast bacilli found was recorded. The time taken to find the first bacillus was also noted.

The results are shown in Table I.

TABLE I

A COMPARISON BETWEEN THREE METHODS FOR PREPARING LIGHTLY INFECTED BOVINE FAECES FOR MICROSCOPICAL EXAMINATION

Sample number	New method			Direct smear			Centrifugation		
	Single organisms	Clumps	Time to find first organism	Single organisms	Clumps	Time to find first organism	Single organisms	Clumps	Time to find first organism
1	70	1	1st field	0	0	—	1	0	2½ min.
2	210	0	1st field	0	0	—	0	0	—
3	5	0	1 min.	4	0	½ min.	0	0	—
4	24	3	1st field	1	0	2 min.	1	0	3 min.
5	19	0	1st field	15	0	1 min.	2	0	1 min.
6	1	0	1½ min.	0	0	—	0	0	—
7	60	0	1st field	4	0	2 min.	6	0	1st field
8	105	2	1st field	3	0	3 min.	4	0	1½ min.
9	14	0	1st field	2	0	3 min.	0	0	—
10	2	0	1¼ min.	0	0	—	0	0	—
11	30	0	¼ min.	0	0	—	0	0	—
12	32	0	1st field	17	0	1st field	4	0	1½ min.
13	30	4	1st field	1	0	4 min.	0	0	—
14	76	3	1st field	17	0	1st field	1	0	4 min.
15	1	0	2 min.	5	0	3 min.	0	0	—
16	32	1	¼ min.	4	0	1st field	2	0	1½ min.
17	1	0	5 min.	0	0	—	0	0	—
Average	41·8	0·8		4·3	0		1·2	0	
Number positive	17			11			8·0		

Using the new method, single acid-fast bacilli were found on all 17 slides and clumps of acid-fast bacilli were found on 6. By the direct smear method, single acid-fast bacilli were found on 11 slides, and by the centrifugation method on 8 slides. No clumps were seen on any of the slides made by methods (2) or (3). The average number of bacilli found was 41.8, 4.3 and 1.2 for methods (1), (2) and (3), respectively. An average of 0.8 clumps was found by the new method. From Table I it will be seen that the time taken to find bacilli by method (1) was much less than that taken using methods (2) and (3).

Trial B. The faeces used in this trial were inoculated with two loopfuls of a pure culture of *Mycobacterium johnei*. In this sample, clumps of acid-fast bacilli predominated.

A hundred consecutive fields were examined on each smear. The number of fields until the first acid-fast bacilli were found and the total number of fields containing acid-fast bacilli were noted.

Results are shown in Table II.

From Table II it will be seen that just under twice the number of positive fields were obtained using the new method than by the direct smear or the centrifugation methods, but in this case there was little difference in the time taken to find the first positive field.

TABLE II

A COMPARISON BETWEEN THREE METHODS OF PREPARING HEAVILY INFECTED FAECES FOR MICROSCOPICAL EXAMINATION

	1	2	3	4	5	Total
New Method						
Number of fields until acid-fasts found ...	5	5	16	3	7	36
Number of fields positive	35	21	23	28	16	123
Direct Smear						
Number of fields until acid-fasts found ...	7	3	27	2	4	43
Number of fields positive	10	11	12	14	20	67
Centrifugation						
Number of fields until acid-fasts found ...	23	7	8	10	5	53
Number of fields positive	14	13	10	10	12	59

Discussion

When examining faeces in which single acid-fast organisms predominated the " straight edge " method was much more efficient than the two other methods used. When examining heavily infected faeces, in which clumps predominated, it was only slightly more efficient.

The " straight edge " smear has continuous good fields along its length which enable the organisms to be more easily seen and differentiated. Use of this method has been found to simplify the examination of large numbers of faeces samples.

Acknowledgments.—This work was supported by an Agricultural Research Council Grant.

The authors are grateful to Dr. W. I. M. McIntyre for advice and criticism.

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(*Concluded at foot of col.* 1 *overleaf*)

The Erythrocyte Sedimentation Rate as a Guide to the Radio-sensitivity of Neoplasms

BY

PETER J. DALTON

Salisbury

SUMMARY.—A method is described by which it is possible to determine the radio-sensitivity of a lesion by a comparison of the erythrocyte sedimentation rate of blood samples taken before and after X-irradiation.

WITH the installation of a therapeutic X-ray unit in this practice it became necessary to become acquainted with the technique of this mode of treatment.

A comprehensive search of available literature revealed, amongst other things, that there was little reported on the effects on the blood constituents of doses of X-rays employed in therapy, although there is a wealth of information available on the blood changes following whole body irradiation (Bacq & Alexander, 1955; Wolff, 1952; Valentine, Pearce & Lawrence, 1952).

It was decided, therefore, to carry out an examination of the blood of patients undergoing X-ray treatment and this included total and differential leucocyte counts, total erythrocyte count, haemoglobin estimation, and the erythrocyte sedimentation rate.

The erythrocyte sedimentation rate was included primarily in the hope that occult metastases of the lesion under treatment might be detected, but as samples were taken before and after irradiation, principally to investigate any changes in the leucocyte content of the blood, a standardised technique was evolved and the erythrocyte sedimentation rate was determined after irradiation, as well as before treatment was given.

It became apparent that a comparison of the erythrocyte sedimentation rate before and after X-ray irradiation gives an indication of the radio-sensitivity of the lesion irradiated.

The erythrocyte sedimentation rate is the rate of fall of the cells in a volume of blood which has been freshly collected and prevented from coagulating by means of the addition of oxalate or citrate anti-coagulants. The test is neither a delicate nor a specific one as the fall is influenced by a variety of factors; such as the temperature in which the test is conducted; physiological—such as pregnancy, sex, and age; and a great variety of pathological conditions—anaemia, tuberculosis, etc.

The phenomenon has been recognised for many centuries, but was first investigated by Fahraeuf (1921). Although many attempts have been made to explain why there should be an increase in the erythrocyte sedimentation rate there would appear to be a variety of factors implicated.

Nichols (1941) made a comprehensive study of this subject, using equine blood with its rapid sedimentation rate in order to accentuate any changes due to faulty technique. Although it has not been possible to employ either the stringent accuracy nor the elaborate equipment advocated by him, meticulous care was exercised to ensure that every investigation was carried out in an identical fashion. In addition, various errors of technique were made on purpose by collecting more blood than was necessary to fill one Westergren tube, dividing it into fractions, and, after putting up one control tube employing the standard technique, committing gross errors of technique on the other fractions and comparing the results. It was found that allowing bubbles to pass up the column of blood and collect at the top of the tube did delay the onset of sedimentation and inaccurate results were obtained also if the tubes were not set up within 30 minutes of collection. The addition of twice the volume of anti-coagulant did not, however, affect the erythrocyte sedimentation rate and this, when dealing with small volumes, is the error most likely to be made. These findings, together with the fact that 14 clinical cases in addition to 6 control cases have given such a similar pattern, permit us to assume that errors in technique have not influenced the findings.

Originally readings were taken as advised by Coffin (1953) at 5-, 10-, and 60-minute intervals, but very little difference was noted between pre- and post-irradiation samples in the first few cases. Due to the fact that treatments and blood examinations usually had to be carried out at night or on Sundays, after the requirements of the practice had been dealt with, the sedimentation tubes were often left where they had been put until the following morning. It was then noticed that there was a considerable difference in the final erythrocyte levels of the pre- and post-irradiation samples. Continuous reading at 10-minute intervals for the first 2 hours, and at various times for the next 4 hours, with subsequent sporadic readings until no further fall was noticed (about 36 hours), were then carried out. It was

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A New Method of Preparing Smears of Bovine Faeces for Microscopical Examination in the Diagnosis of Johne's Disease—*Concluded.*

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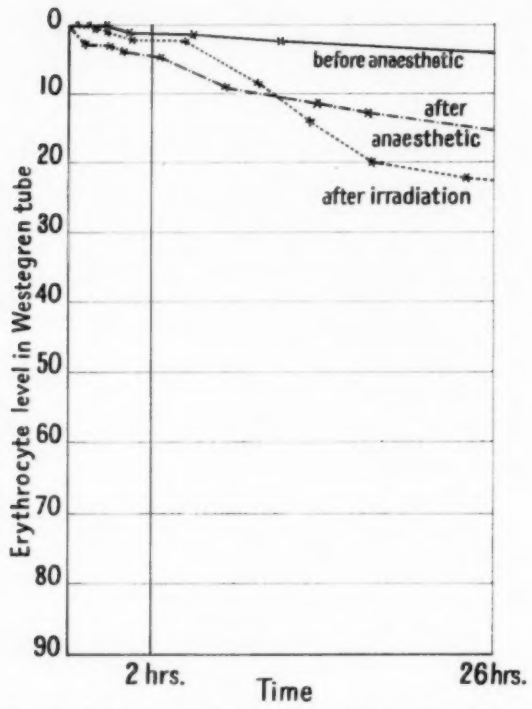

FIG. 1.—Effect on erythrocyte sedimentation rate of anaesthesia and irradiation of large radio-resistant lesion.

found that there was often a delayed drop commencing at about the second hour (see Fig. 1).

It was noticed also that the administration of barbiturate anaesthetics had a slight effect on the prolonged erythrocyte sedimentation rate and this was investigated in 6 apparently healthy dogs of varying ages and sexes which were brought in for minor clinical procedure, such as the removal of grass seeds from ears. It was noted also that irradiation of *any* lesion caused an increase in the erythrocyte sedimentation rate.

Fig. 1 illustrates also the erythrocyte sedimentation rate changes which occurred when a hypertrophic lesion was irradiated. This case (Plate I) was a spaniel whose ear had been resected to alleviate otitis externa.

The grossly enlarged rougae of the aural canal had continued to proliferate and X-ray therapy was given to stop seborrhoea which was present. Although a large mass of tissue was irradiated, it was hypertrophic and, therefore, radio resistant, and consequently the change in the erythrocyte sedimentation was slight.

It would seem reasonable to postulate that the changes in the erythrocyte sedimentation rate which occur following irradiation of such tissues are due to the effect of the X-rays on radio-sensitive cells such as leucocytes, epithelial and endothelial cells which are to be found in all tissues.

The changes in the erythrocyte sedimentation rate which occur following the irradiation of pathological lesions are much more pronounced, and the degree of the increase in the delayed erythrocyte sedimentation rate appears to be proportional to the amount and radio-sensitivity of the tissues exposed to irradiation.

The irradiation of Case 38 (Plate II), when a large amount of highly radio-sensitive tissue was present, produced a greatly increased erythrocyte sedimentation rate. Subsequent irradiation when the lesion was visibly regressing (Plates III and IV) produced progressively smaller sedimentation (see Fig. 2). The tumour was an adenocarcinoma of the circumanal glands and the irradiations given were 741 roentgen at a half-value layer of 1.35 aluminium at a dosage rate of 74 roentgens per minute.

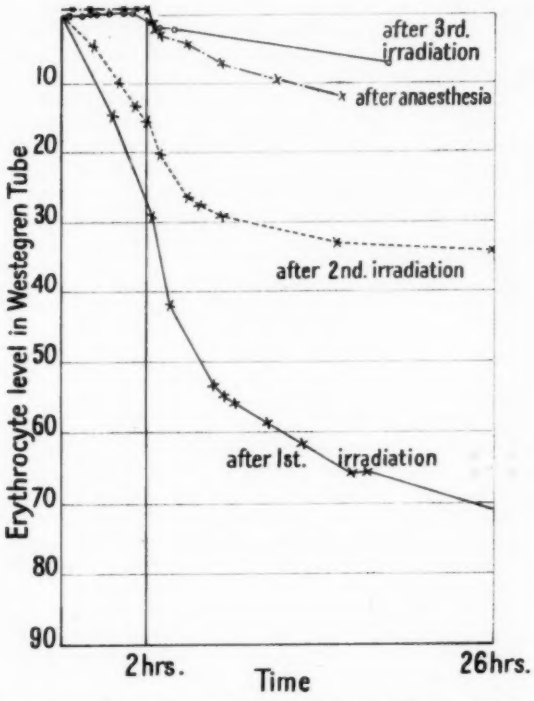

FIG. 2.—Effect on erythrocyte sedimentation rate after irradiation of large radio-responsive lesion.

Attempts to Determine Causes of Increased Erythrocyte Sedimentation Rate

The examination of a thin film of blood taken after irradiation did not reveal rouleaux formation or agglutination. Neither was there any difference in the total erythrocyte count and haemoglobin concentration of pre- and post-irradiation samples to explain the increase of sedimentation on the grounds of anaemia. By means of separating the cells and plasma of pre- and post-irradiation samples taken from a dog which exhibited an increased erythrocyte sedimentation rate following irradiation of a radio-sensitive lesion, and by manipulating the fractions by washing, etc., and then reconstituting the bloods in various ways, an attempt was made to determine

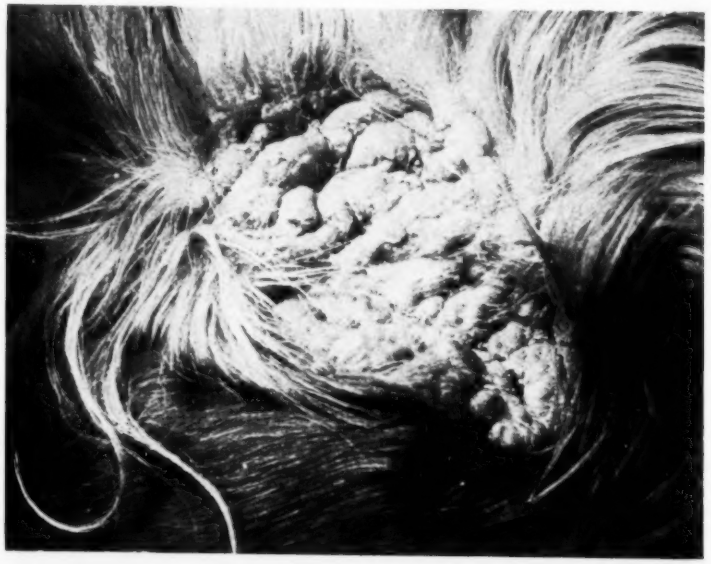

PLATE I.—Hypertrophy of aural rougae.

PLATE II.—Adenocarcinoma of circumanal glands. (Case 38.) Before 1st irradiation.

PLATE III.—Before 2nd irradiation.

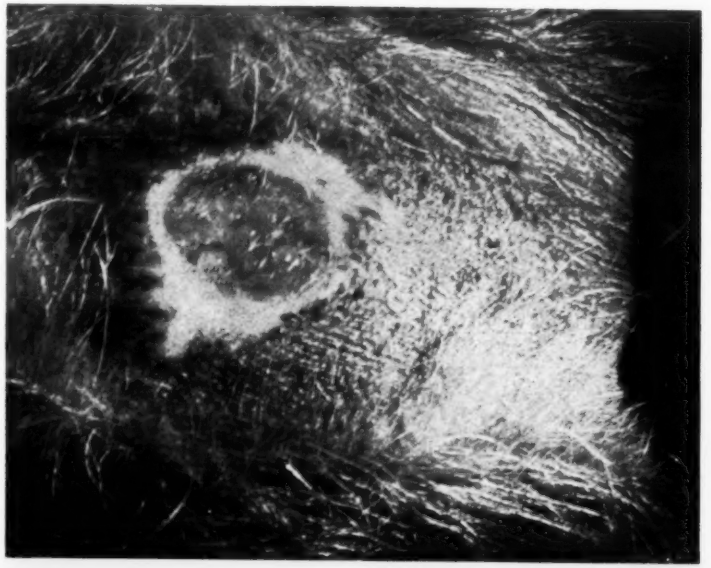

PLATE IV.—Before 3rd irradiation.

TABLE

ERYTHROCYTE SEDIMENTATION READINGS OBTAINED BEFORE AND AFTER IRRADIATION OF VARIOUS TYPES OF LESIONS

Case No.	Animal. Age. Sex.	Lesion	Date	Dose r.	E.S.R. reading Time/hours 1	2	12	24	Response
60	Goodman, Alsatian, 9 years, male	Swelling on right side face	12.6.56	736	1 2	2 3	5 12	6 20	Nil
67	Way, spaniel, 3 years, female	Large soft non-malignant swelling on right lower lid	7.10.56	355	4 3	5 5	15 15	20 22	Nil
66	Lindley, retriever, 10 months, male	Epithelioma on left fore wrist (small)	26.9.56	741	2 1	4 4	11 20	17 32	Good
			5.10.56	741	1 1	1 4	4 10	6 19	Good
38	Cook, mongrel, 10 years, male	Large circumanal adenoma	10.1.56	1,482	1 22	1 28	8 61	12 69	Good
			17.1.56	2,223	— 5	— 16	— 31	— 33	Good
			15.2.56	1,111	— 0	— 0	— 9	— 12	Good
54	Blaine, retriever, 9 years, male	Large undifferentiated epithelioma of thigh	13.4.56	1,516	2 2	4 5	14 20	18 31	Good
			31.5.56	718	2 2	5 5	14 20	19 37	Good
56	Hicks-Beach, terrier, 7 years, female	Seborrhoea and hypertrophy of aural rougae	8.5.56	741	2 10	4 18	7 42	10 —	Seborrhoea cleared
			26.6.56	570	2 0	4 2	13 7	— —	Nil
58	Kelly, golden retriever, 10 years, male	Histiocytoma of nose with infiltration of inflammatory cells	17.5.56	741	1 8	2 15	4 50	5 68	Good
			24.5.56	1,111	1 2	6 9	32 48	55 74	Good
69	Gillings, sheepdog, 14 years, male	Spindle celled sarcoma of upper jaw	7.10.56	958	— —	— 29	— —	— 71	Good
			21.10.56	971	0 7	0 —	8 17	10 33	Good
			28.10.56	1,224	0 3	0 9	— 60	6 —	Good
76	House, labrador, 8 years, male	Adenoma of circumanal glands	18.11.56	294	4 5	7 12	14 39	36 71	Good

r. = roentgen.

if either fraction, *i.e.* cells or plasma on its own, was responsible for the increased erythrocyte sedimentation rate. The results, however, were not conclusive. This portion of the work was kindly carried out for me by Dr. E. Boulter, for whose interest and advice I am greatly indebted.

It would appear that the alteration of the erythrocyte sedimentation rate is due to the liberation into the blood stream of some substance from radiosensitive cells after they have been exposed to X-rays.

The Table shows the very slight increase in the erythrocyte sedimentation rate following irradiation of non-radio-responsive lesions (Cases 60, 67, 56) when compared with the sedimentation rate which occurs following irradiation of radio-responsive lesions.

The figures for Case 56 are of particular interest. It is suggested that the increase in erythrocyte sedimentation rate following the first irradiation is due

(*Concluded at foot of col.* 1 *overleaf*)

Long Distance Transportation of Fowl Semen by Air

BY

P. E. LAKE

A.R.C. Poultry Research Centre, Edinburgh

H. SCHINDLER

Agricultural Research Station, Rehovot, Israel

AND

F. H. WILCOX

Department of Poultry Husbandry, University of Maryland, Baltimore, U.S.A.

SUMMARY.— Fowl semen was flown across the Atlantic from the University of Maryland, U.S.A., to Israel and Scotland. An account is given of the method used to dilute and transport the semen, and the fertility results obtained during the first week after insemination.

FOWL semen has proved particularly difficult to store *in vitro* and from research in progress it seems possible that part of the failure is due to a lack of fundamental knowledge of the chemical and physical nature of the semen. Consequently when spermatozoa have been held *in vitro* they have been placed in unfavourable fluid environments with regard to composition, osmotic pressure, *p*H and buffering capacity. Many synthetic media and biological fluids have been used empirically either separately or as a mixture, for fowl semen diluents, *e.g.* Tyrode, Ringer, Ringer-Locke, glycine, yolk-phosphate, yolk-citrate solutions, albumen and milk. All of these have either a different osmotic pressure to fowl seminal plasma or a poor buffering capacity; with the exception of a single report of fair success using milk with either albumen or citrate-glucose-saline as a diluent and storing semen for 24 hours (Polge, 1955), none has proved consistently satisfactory for storage purposes (Schindler, Weinstein, Moses & Gabriel, 1955; Takeda, 1954).

Wilcox and Shaffner (1957) showed that simple phosphate buffers, with freezing point depression measurements in the range −0.607° to −0.639° C., to a certain extent preserved the fertilising capacity of fowl spermatozoa *in vitro*; subsequently using one of the buffer solutions with added antibiotics, they obtained promising fertility results after storing semen for 24 hours at 10° C. (Wilcox & Shaffner, 1958). As an interesting experiment it was decided to use the latter method in an attempt to transport semen long distances by air from Maryland, U.S.A. to Israel and Scotland, and the present paper reports the fertility results obtained after such a venture.

Method and Material

Semen was collected by the customary method from 8 Flightless (90 per cent. New Hampshire and 10 per cent. White Leghorn blood) and 8 Broiler (Barred Columbian) strain males kept at the University of Maryland, U.S.A. It was immediately diluted ten-fold with a buffer containing antibiotics [Na_2HPO_4, (16.34 g.), Na H_2PO_4. H_2O (5.16 g.), oxytetracyline* (100 mg.) and dihydrostreptomycin (1 g.) per litre] and placed in plastic vials. Then a can containing ice was wrapped in cotton wool and placed in a paper bag. The vials were placed around the outside of the bag, and the whole put in an insulated container (Fig. 1). This procedure was devised at Maryland to maintain a temperature of 10° C. Samples of semen were taken to the nearest airport and flown to Scotland and Israel. Upon

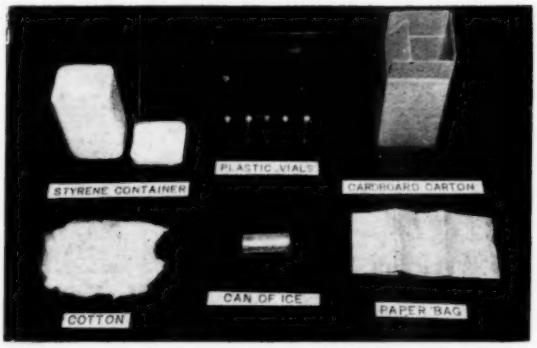

FIG. 1.—Materials used in packaging semen.

* Terramycin.

The Erythrocyte Sedimentation Rate as a Guide to the Radio-sensitivity of Neoplasms—*Concluded.*

to the fact that at that time there were present large numbers of inflammatory cells and hyperplasia of the sebaceous glands of the aural integument, both of which are radio-responsive. At the second irradiation the inflammation and hyperplasia had subsided and there was therefore little increase in the erythrocyte sedimentation rate.

It is regretted that there are some gaps in the Table, but the work was carried out during the course of everyday general practice and it was not always possible to take as many blood samples or readings as one would have wished.

Acknowledgments.—I would like to record my most sincere thanks to Dr. E. Boulter and to Mr. Roberts of Newton Victor for their constant help and encouragement, and to the staff of the R.C.V.S. library for obtaining for me the numerous publications which had to be perused.

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TABLE I

FERTILITY RESULTS OBTAINED WITH TRANSPORTED SEMEN

Treatment of semen	Number of hens inseminated	Number of hens producing one or more fertile eggs	Number of eggs set	Per cent. fertility (eggs laid 1st week)	Per cent. hatchability
Transport to Israel					
1. Fresh, undiluted semen used at Maryland ...	10	9	47	81	94
2. Stored 35 hours at 10° C. and treated as transported semen at Maryland					
(*a*) Hen without egg in uterus	10	4	41	17	67
(*b*) Hen with egg in uterus	10	4	40	18	50
3. Transported semen	27	18	125	38*	not recorded
Transport to Scotland					
1. Fresh, undiluted semen used at Maryland... ...	10	10	46	94	95
2. Stored 43 hours at 10° C. and treated as transported semen at Maryland					
(*a*) Hen without egg in uterus	10	3	45	16	71
(*b*) Hen with egg in uterus	9	2	38	8	100
3. Transported semen	20	13	100	36†	81

* 49 per cent. from 8 hens with hard-shelled egg in uterus ; 33 per cent. from 19 hens without hard-shelled egg in uterus.
† 25 per cent. from 8 hens with hard-shelled egg in uterus ; 43 per cent from 12 hens without hard-shelled egg in uterus.

arrival at the respective laboratories in the two countries the semen was centrifuged (1200 RCF) for 10 minutes and the supernatant poured off. The spermatozoa were resuspended in enough phosphate buffer, containing no antibiotics but 2.5 mg. per ml. of fructose, to bring them to their original semen volume. Hens were inseminated deeply into the vagina with 0.1 ml. semen by the customary procedure. As the semen samples arrived at their destinations in the early morning some hens had eggs in their uterii and note was taken of these cases in the analysis of fertility results.

The following types of semen from the same males were inseminated in two control tests at Maryland : (1) 0.1 ml. undiluted semen and (2) 0.1 ml. semen diluted and stored approximately for the same time taken for the long distance transport.

Results

The fertility results are summarised in Table 1. The total elapsed time for collection of semen to insemination was 38 hours to Israel and 37 hours to Scotland. The temperature of the semen upon arrival was 11° C. and 10.8° C. in the respective countries.

Discussion

The fertility results obtained with semen which had been packaged and sent on journeys lasting more than 30 hours were encouraging, and give added stimulus for more basic work on the physiology of chicken semen, and consequently better methods of handling the material. Lake (unpublished observations), using Ramsay's (1949) micro-method, estimated the freezing point depression of fowl seminal plasma to be between −0.59° and −0.6° C. and Schindler *et al.* (1955) gave a value of −0.64° C. for whole semen. Both these values are higher than that for bull seminal plasma, −0.53° C. (Rothschild & Barnes, 1954), which could indicate that the plasma of the fowl is different in composition from that of the bull, and in this respect should warrant special attention when the dilution and storage of fowl semen is under consideration. Recently, very promising fertility results were obtained using semen which had been stored for 24 hours (Lake, 1958); the composition of the diluent was based on a partial chemical analysis of protein- and lipid-free seminal plasma. The results of the present test were obtained by using a method of diluting and storing semen derived from observations on the best *p*H, buffering capacity and osmotic pressure required of a phosphate buffer solution for preserving the fertilising capacity of fowl spermatozoa (Wilcox & Shaffner, 1957, 1958).

The present work and its extension has many implications of practical importance with regard to the transport of poultry. There is probably less chance of introducing disease from one country to another, as compared to the shipment of hatching eggs, chicks and adult birds. The semen shipped was diluted with a solution containing the antibiotics. oxytetracycline and dihydrostreptomycin. The cost is less. A quart of diluted semen could be shipped fairly inexpensively and would be enough to inseminate 1,000 hens. At 37 per cent. fertility. 2,000 chicks could be hatched from a single shipment. This procedure offers a faster method of introducing high production stock where it is desired to eventually cross it to native chickens. By this method the latter can be inseminated with semen from high production stock, which saves a year in breeding programmes for improving native stock.

The future prospects for world-wide transport of chicken semen appears bright provided the semen could be allowed through customs barriers and satisfy animal health controls. Improved fertility should be obtained with either better cross-country airway connexions or faster planes to shorten the time in transit, and with better methods of handling semen *in vitro* which should arise from extended

Current Literature

ABSTRACTS

Food Poisoning in England and Wales, 1957. *Monthly Bull. Min. of Health* (1958). **17.** 252.

In England and Wales in 1957, 7,071 incidents of food poisoning occurred; this figure was made up of 473 general outbreaks, 501 family outbreaks, and 6,097 sporadic cases. There was a reduction on preceding years.

Salmonellae were responsible for 95 per cent. of incidents due to recognised pathogens, and *Salmonella typhimurium* was present in 66 per cent.

Thirty-six fatal cases were recorded, of which 32 were due to Salmonellae, 3 to *Cl. welchii*, and one to an unknown cause. Maximum incidence occurred in the summer months reflecting the close relation between atmospheric temperature and the growth of bacteria in food.

A wide variety of food was implicated: fish, meat, sweetmeats, eggs, cheese, vegetables, and fruit. Poisoning by made-up meats was associated mostly with *Cl. welchii*. Of special interest were 17 outbreaks associated with canned peas (16 staphylococcal and one unknown).

No outbreaks associated with milk were reported.

Cl. welchii was identified with canteen outbreaks, but in hospitals most outbreaks were associated with Salmonellae, cross infection having played a part.

Contamination with Salmonellae was widespread in most of the imported egg products. Cleanliness and heat treatment of the egg products would eradicate this source of infection, but it is impossible at present to estimate the effect that pasteurisation of egg products would have on salmonellosis as a whole.

Pig meat remains, as in the past, a frequent source of infection by Salmonellae. Infected but symptomless pigs delivered to an abattoir might infect 90 per cent. of the pigs with which they were in contact in the pre-slaughter pens, and once infection was introduced into the abattoir it spread to the cutting-up rooms. Salmonellae isolated from live and slaughtered pigs were of the same types as those found in food handlers (mostly symptomless excretors) and in other human cases living in the area served by the abattoir. It seemed probable that individual pigs were infected on the farms, and when brought to the abattoir might infect others in the lairages. Limiting the duration of stay in the lairages, and prevention of overcrowding, might substantially reduce the proportion of animals found infected at slaughter.

Fish-meal, bones, and dried blood used in the preparation of fertilisers and animal feeding-stuffs were contaminated by Salmonellae up to 40 per cent. of samples taken. Animals fed on such material remained well, but their gut contained the Salmonellae which might spread into the abattoir and carcase meat. Heat treatment of imported feeding-stuffs from backward tropical countries is therefore indicated. W. T. F.

Long Distance Transportation of Fowl Semen by Air —*Concluded.*

research on the nature of fowl semen, the behaviour of the spermatozoa, and indeed the whole subject of reproduction of the bird.

Acknowledgments.—The shipment of semen to Israel was made possible by the production of a certificate from a federal veterinarian in the U.S.A., stating that the stock at Maryland was free of disease communicable by semen. The help of Drs. S. Bornstein, E. Moses and I. Gabriel is acknowledged.

The part of the experiment in Scotland was made possible through the granting of a licence solely for scientific purposes by the British Ministry of Agriculture, and the Department of Agrigulture for Scotland. The trial was carried out at an official laboratory under strict precautionary safeguards which included the destruction not only of all progeny chicks but of all hens involved. The authors are very grateful for the above mentioned permission, and to Dr. J. E. Wilson, Director of the Ministry of Agriculture's Veterinary Research Laboratories, Lasswade, Scotland, for arranging facilities to house and isolate hens for insemination purposes and for collecting fertility and hatchability records.

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Studies on Intravertebral Disc Protrusion in the Dog. VAUGHAN, L.C. (1958). *Brit. Vet. J.* **114.** 12. 458-63.

In this, the fourth article of the series, the author deals with the treatment of the condition. Medical treatment consists essentially of good nursing together with the relief of pain by means of sedatives and analgesics. In cases of complete paraplegia it is thought that vitamin B_1 may play a useful part in improving the metabolism of the nervous tissue.

Two methods of surgical treatment are described and discussed, namely laminectomy and disc fenestration. The former procedure is considered too hazardous to be recommended, involving as it does a direct opening into the spinal canal with the consequent risk of damage to the cord.

By means of the fenestration operation it is possible to prevent a further extrusion of the nucleus and it allows a release of pressure which during spinal movements has been acting on the protrusion. It is chiefly indicated in dogs with symptoms of pain and/or limited locomotor dysfunction which have not responded to conservative treatment.

News and Comment

HAZARDS OF EXPOSURE TO RADIOACTIVITY

For the greater safety of veterinary surgeons using X-rays and other radioactive sources, the Ministry of Agriculture draws the attention of the profession to the Code of Practice for the Protection of Persons exposed to Ionizing Radiations, prepared by the Ministry of Health and published by H.M. Stationery Office, price 8s. A copy of the Code is available at B.V.A. Headquarters, and in the Royal College of Veterinary Surgeons' Library.

THE MEAT (STAINING AND STERILISATION) REGULATIONS, 1959

From April 1st, all meat which is unfit for human consumption will have to be sterilised before being sold by retail. The only exception is for unfit meat supplied to zoos, menageries, and mink farms, but such establishments must obtain their meat direct from slaughterhouses or knackers' yards who will have to stain it green before sale.

This is one of the main provisions of regulations which were laid in Parliament on January 9th by the Ministers of Agriculture and Health. The regulations will not apply to retail sales alone. When they come into force, it will be an offence for any unfit meat to leave a slaughterhouse or for any meat to leave a knackers' yard unless it has been stained or sterilised. Importers of "unfit" meat will also have to stain or sterilise it as soon as possible after it comes into the country if this has not already been done; and butchers or other dealers in meat for human consumption will have to do likewise with any meat which may become unfit whilst in their possession.

THE STEELE-BODGER MEMORIAL PRIZE

This prize was created by the fund established in 1953 to perpetuate the memory of the late Harry Steele-Bodger. It takes the form of a travelling scholarship or a contribution to a tour of study abroad. In addition, applications for grants of small amounts to enable practising members of the profession to make short study tours abroad receive sympathetic consideration.

Members of the Royal College of Veterinary Surgeons are eligible, together with final-year students of any of the university veterinary schools in the United Kingdom and Eire.

The closing date for applications is July 31st, and further particulars may be obtained from the General Secretary of the B.V.A.

NEW R.V.C. PROFESSOR

As briefly announced in our issue of January 3rd, Dr. J. A. Laing, Reader in Veterinary Science at the Bristol School, has been appointed to the Courtauld Chair of Animal Husbandry and Veterinary Hygiene at the Royal Veterinary College in the University of London.

Dr. Laing qualified at Edinburgh in 1941 and was Aleen Cust Scholar in 1941-3. He then studied under Dr. John Hammond at the School of Agriculture, Cambridge, and was awarded the Doctorate in Philosophy in 1944. For the next 2 years he worked on infertility problems under the direction of Sir Thomas Dalling, and joined the Ministry's service in 1946, becoming Assistant Veterinary Investigation Officer at Cambridge.

When the Bristol School was founded Dr. Laing was the first teacher to be appointed after the late Professor Blakemore, whom he assisted in the development of the School, also initiating research work in many fields of animal husbandry and breeding. He was appointed Senior Lecturer in Veterinary Medicine in 1951, and Reader in Veterinary Science in 1957. Dr. Laing has both worked and travelled abroad, having studied in the Royal Veterinary and Agriculture School, University of Copenhagen, as the first Churchill Fellow, and having been F.A.O. Representative in the Dominican Republic (1957-8) while on leave from the University of Bristol.

As our readers will know, his name is associated with many publications on animal breeding and fertility, and he is the author of a standard text-book on these subjects. The staff of the Royal Veterinary College will welcome in him a notable scientist, and his colleagues everywhere will wish him a long and distinguished tenure of his first University Chair.

THE ASSOCIATION OF VETERINARY STUDENTS

The annual conference of the Association of Veterinary Students will be held in London and Cambridge on Friday, Saturday, and Sunday, January 23rd to 25th. A wide ranging and interesting programme, of which some details are published in our Notices column includes a closed-circuit television surgery demonstration and a symposium to which the contributors will be Mr. R. S. Marshall, C.B.E., adviser on animal health to the Colonial Office, Mr. L. Guy Anderson, Dr. N. S. Barron, and Dr. A. W. Stableforth, director of the Ministry's veterinary laboratories.

The official opening ceremony will be performed by the Minister of Agriculture and Dr. R. E. Glover, Principal of the Royal Veterinary College (where the first two days' meetings will take place) will reply.

PROFESSOR SCHOFIELD IN KOREA

Members of the profession who know and esteem Professor Frank W. Schofield, the distinguished Canadian pathologist, will be interested to know that he was recently a guest at the tenth birthday celebrations of the Korean republic. A special message of welcome to him was sent by President Syngman Rhee, at whose invitation he attended the ceremony. He addressed a public meeting, speaking partly in Korean, and it was recalled that he had been a champion of the Samil independence movement for many years.

He will remain in Korea for six months, and is to lecture in pathology at the Seoul National University College of Veterinary Medicine.

MR. ROBERT M. SMITH, J.P.

Mr. A. L. Wilson writes:—

Many members of the profession will learn with regret of the death of Mr. R. M. Smith at his home, Thornwood, in Galston, Ayrshire, on January 5th. Mr. Smith, who was of farming stock, graduated from Glasgow Veterinary College on July 21st, 1921, and after a period of general practice in England, returned to Ayrshire to take over the Galston practice from Mr. George Scade. At this time a great deal of attention was being paid to tuberculosis of dairy cattle and Mr. Smith, together with colleagues from the Ayrshire Division and Ayrshire County Council, was very active in the campaign to test all the cattle.

Being a farmer's son Mr. Smith's interest in agriculture was deep and abiding. He was a director of Kilmarnock Farmers' Society and also of the Sorn Parish Agricultural Society. He was also President of the Loudoun and Galston Agricultural Association and for several years in partnership with his brother, Mr. John Smith of Auchenbay, Mauchline, he owned a Clydesdale stud and sat on the Council of the Clydesdale Horse Society. A few years ago Mr. Smith acquired the well-known dairy farms of Holehouse and Sornbeg, both of which are in the Galston area.

He devoted a great deal of time to veterinary matters and served at various times as President of the Ayrshire and West of Scotland Divisions, and also as a Council member of the B.V.A. He was also a Council member of the Scottish Branch up to his death.

Of a quiet and unassuming nature, "Bob" Smith possessed a great wealth of practical experience which benefited many students who "saw" practice" in Galston. Accompanied by his wife he regularly attended the annual conferences of the B.V.A., and the various veterinary social functions in the West of Scotland. He is survived by his wife, a son who is a member of the veterinary profession, and a married daughter.

Among a very large number of friends who gathered at Galston cemetery on January 7th to pay their last respects were the following members of the profession: Messrs. H. Gillmor, F. McD. McKenzie, D. Moodie, A. A. Young, J. M. Robertson, and W. G. Johnston representing the Ayrshire Division; A. Thomson, H. Begg, A. S. Chapman, and D. T. Bickerton representing the West of Scotland Division; S. Jennings representing the Scottish Branch; J. Nicholson and Miss E. V. H. Porter representing the Animal Health Division; Dr. P. S. Blackburn representing the Hannah Dairy Research Institute; A. L. Wilson representing the President of the B.V.A., and J. G. Gordon a former assistant.

PERSONAL

Mr. C. Jones, M.R.C.V.S., Veterinary Officer at Ruthin, resigned from the Ministry as from December 31st, 1958.

Birth

GROSE.—On December 17th, 1958, at Glen Cottage, Boughton, Northants, to Mary (*née* Green, M.R.C.V.S.), and Bill Grose, a son, brother for Gabrielle.

COMING EVENTS

January

21st (Wed.) 10th Anniversary Dinner-Dance of the Centaur Society at the Berkeley, Bristol.

23rd, 24th and 25th (Fri., Sat. and Sun.). Annual Conference of the Association of Veterinary Students in London and Cambridge.

Meeting of the Warwickshire Veterinary Club in the Regent Hotel, Leamington Spa, 8 p.m.

28th (Wed.). Winter Meeting of the North of Scotland Division in the Marcliffe Hotel, Aberdeen, 2.30 p.m.

General Meeting of the Sussex Veterinary Society in the Old Ship Hotel, Brighton, 2.30 p.m.

General Meeting of the Southern Counties Veterinary Society in the Grand Hotel, Bournemouth, 7.45 p.m.

30th (Fri.). Annual Ball of the Royal (Dick) School of Veterinary Studies in the Assembly Rooms, George Street, Edinburgh, 9 p.m.

Annual General Meeting of the Yorkshire Veterinary Society in the Hotel Metropole, Leeds, 1, 2.30 p.m.

Annual General Meeting of the North of Ireland Veterinary Association in the Faculty of Agriculture, Elmwood Avenue, Belfast, 7 p.m.

February

4th (Wed.). Meeting of the West of Scotland Division at the Veterinary Hospital, Bearsden Road, Bearsden, Glasgow, 2.30 p.m.

11th (Wed.). Annual General Meeting of the Society for the Study of Animal Breeding at the Royal Society of Medicine, 1, Wimpole Street, W.1.

7th Annual General Meeting of the Society for the Study of Animal Breeding, in the West Hall, R.S.M., 1, Wimpole Street, London, W.1, 11.30 a.m.

March

20th, 21st and 22nd (Fri., Sat. and Sun.). Conference of the British Small Animal Veterinary Association in London.

BRITISH VETERINARY ASSOCIATION QUARTERLY MEETINGS IN JANUARY

Tuesday, January 20th, 1959—At No. 7, Mansfield Street

5.30 p.m. Finance Sub-committee.

Wednesday, January 21st, 1959—At No. 7, Mansfield Street

10.00 a.m. Veterinary State Medicine Committee.

11.30 a.m. Home Appointments Committee.

2.00 p.m. Small Animals Committee.

Thursday, January 22nd, 1959—At No. 7, Mansfield Street

10.00 a.m. Parliamentary and Public Relations Committee.

11.30 a.m. Farm Livestock Committee.

2.15 p.m. General Purposes and Finance Committee.

Friday, January 23rd, 1959—At The Connaught Rooms, Gt. Queen Street, W.C.2

11.00 a.m. Council Meeting.

ADDRESSES OF DISEASE INFECTED PREMISES

The list given below indicates, first the county in which are situated the premises on which disease has been confirmed, followed by the postal address and date of outbreak.

Anthrax

Berks. Moor Mill Farm, Uffington, Faringdon (Jan. 6).

Durham. S. Johns Chapel, Bishop Auckland (Jan. 12).

Lancs. Copper Farm, Whitehill Road, Marton, Blackpool (Jan. 12).

Yorks. Ash House Farm, Catterick Camp Road, Catterick (Jan. 12).

Fowl Pest

Dorset. Forest Deer, Gillingham (Jan. 6); Forest Farm, Gillingham (Jan. 11).

Essex. Ramsey Island Farm, Steeple, Southminster (Jan. 6); The Mill, Wakes Colne, Colchester (Jan. 7); Griffin Wood, Copped Hall, Epping; Stocks Farm, Boreham, Chelmsford (Jan. 8); Rough Hill Farms, Rough Hill, E. Hanningfield, Chelmsford (Jan. 9).

Lancs. Back o' th' Moss Farm, Unsworth, Bury (Jan. 7).

Lincs. Bank House, Ruskington Fen, Billinghay (Jan. 8); Chapel Lane, Anwick, Sleaford (Jan. 9); Anwick, Sleaford (Jan. 12).

Middx. Grove Farm, Batchworth Lane, Northwood (Jan. 12).

Norfolk. The Bungalow, High Street, Wicklewood, Wymondham (Jan. 7).

Sussex. Yew Tree Farm, Westfield, Battle (Jan. 9).

Swine Fever

Bucks. Ivy Hall Farm, Cranfield, Bletchley (Jan. 8).

Ches. Long Rake Farm, Newton, West Kirby, Wirral (Jan. 8).

Denbigh. Commonwood Farm, Commonwood, Holt, Wrexham (Jan. 9).

Derby. Thorntree Farm, Middleton by Youlgreave, Bakewell (Jan. 6).

Devon. Goffins Farm, Stoke Hill, Exeter (Jan. 7); Bow Bridge House, Ashprington, Totnes (Jan. 8); Mosshayne Farm, Broadclyst (Jan. 9).

Durham. Tile Shed Lane Piggeries, Cleadon, South Shields (Jan. 8).

Essex. Bridge Farm, Marks Tey, Colchester (Jan. 6); Woodrolfe Farm, Tollesbury, Maldon (Jan. 9); Woodlands Farm, Romford Road, Chigwell Row (Jan. 12).

Glos. Model Pig and Poultry Farm, Marsh Lane, Easton in Gordano, Bristol (Jan. 12).

Lancs. Little Hey Farm, Moss Side, Formby, Liverpool (Jan. 7); Sniggery Farm, Hightown, Liverpool, 23 (Jan. 9); Beech Farm, Eaton Road, West Derby, Liverpool, 12 (Jan. 10).

Leics. Fosse Farm, Sapcote (Jan. 6).

Norfolk. Ferriers Allotments, High Mill Road, Cobholm, Great Yarmouth; Corner Farm, Forncett St. Peter, Norwich (Jan. 6); Blackhorse Public House, Flordon, Norwich (Jan. 7).

Suffolk. Windycross, Waldringfield, Woodbridge; High House Farm, Claydon, Ipswich (Jan. 9).

Warwicks. Piggeries, Napton Road, Allotments, Leamington Spa (Jan. 8); Charity Farm, Baxterley, Atherstone (Jan. 12).

Wilts. Holt Road Farm, Bradford-on-Avon (Jan. 7); Jumps Farm, Brickley Lane, Devizes (Jan. 9).

Worcs. Highfield Farm, Headley Heath, Kings Norton, Birmingham, 30 (Jan. 9).

Yorks. Tanner Farm, Littletown, Liversedge (Jan. 6).

LIVER FLUKE OUTBREAK IN WALES

It is reported that veterinary surgeons are fighting a major outbreak of liver fluke and black disease among sheep in Montgomeryshire. Some farmers report losses up to 60 per cent. One veterinary surgeon alone is said to have conducted *post-mortem* examinations on nearly 200 sheep. The heavy infestation is considered to be a result of excessively wet weather which has led to a great multiplication of the snail.

DISEASES OF ANIMALS ACT, 1950—GREAT BRITAIN

Summary of Returns of Confirmed Outbreaks of Scheduled (Notifiable) Diseases

Period		Anthrax	Atrophic Rhinitis	Foot-and-Mouth	Fowl Pest	Sheep Scab	Swine Fever
16th to 30th Nov., 1958		4	—	—	25	—	39
Corresponding period in	1957	9	—	10	81	—	33
	1956	21	—	11	98	—	33
	1955	54	—	1	110	—	57
1st Jan. to 30th Nov., 1958		143	5	116	667	—	1,146
Corresponding period in	1957	299	8	135	897	—	885
	1956	1,218	11	146	771	—	700
	1955	721	6	7	624	—	1,131

Letters to the Editor

The views expressed in letters addressed to the Editor represent the personal opinions of the writer only and their publication does not imply endorsement by the B.V.A.

SCIENTIFIC

Attempts to Demonstrate Antibodies in Scrapie Disease

Sir,—The aetiology of scrapie disease is undecided, but there is good evidence that the disease is caused by a transmissible agent and can be maintained by serial passage in sheep and goats (Gordon, 1957). The agent has several of the attributes of a virus, for example it is filtrable (Wilson *et al.*, 1950); however, reports indicate that the agent is unusually resistant to physical and chemical treatment, surviving 100° C. for 30 minutes (Wilson, 1954) and exposure to 0.35 per cent. formalin over a period of at least 3 months (Gordon, 1946). Although it is now generally accepted that the agent is transmissible and has several of the properties found in viruses, previous workers have failed to demonstrate the presence of specific antibody. Thus, complement-fixation tests have been conducted by Gordon and by Wilson (Gordon, 1958) using sera from scrapie sheep and saline brain extracts, or cerebrospinal fluid, as antigens; no antibodies were detected. Matthews (1958) used the Ouchterlony gel-diffusion plate technique (Ouchterlony, 1948) in an attempt to demonstrate antibodies. His antigens, which consisted of brain tissue and cerebrospinal fluid from scrapie goats, were tested against sera and cerebrospinal fluid from affected goats; in all cases negative results were obtained.

Serological tests of several types are in use at this laboratory and, since scrapie material is also available, tests were made in an unsuccessful attempt to detect antibodies in several cases of scrapie. A summary of the procedures, and results obtained, is given below as an appendix.

These negative results do not prove that there is no antibody response to the scrapie agent. The sera tested may not have contained antibody, and the antigens used may have been unsuitable. The results suggest, however, that demonstration of an antibody response in scrapie is unlikely to follow the use of standard techniques applied to a limited number of sera.

APPENDIX

Antigens. These were of two main types: cerebrospinal fluid obtained from advanced clinical cases of scrapie in artificially infected goats, and saline extracts of brains from artificially infected sheep at an earlier, though clinical, stage of the disease. The brain extracts were made from a pool of medulla, mid-brain, and frontal cortex by grinding the tissue with sand in a mortar, saline being added; the suspension was then frozen and thawed five times, centrifuged, and the supernatant used as antigen.

In addition an antigen was prepared from spleen by grinding, suspending in saline, centrifuging, and using the supernatant.

Sera. The sera were obtained from artificially infected goats in an advanced clinical state, from similarly infected sheep at an early clinical stage, and from naturally infected sheep in an advanced clinical state. The sera were used fresh or after storage for not more than 2 months in the frozen state; the majority of the sera were used in more than one type of test.

Serological Tests. Ten different types of tests were used:—

1. *Tanned Erythrocyte Haemagglutination Test.* The technique was similar to that described by Boyden (1951). Seven tests were conducted; the antigen used was derived from goat cerebrospinal fluid in 4 tests and from sheep brain in 3 tests; the sera used for each test were from 3 scrapie sheep, 3 normal sheep, 2 scrapie goats, 3 normal goats. More than one dilution of antigen was used in each test; it was necessary to dilute the antigen considerably to avoid non-specific agglutination which was particularly common when using sheep brain antigen. No difference between scrapie and normal sera was observed.

2. *Middlebrook Dubos Test.* The technique was similar to that described by Boyden. Two tests were conducted, the antigen used being of the brain extract type. No differences were observed between sera from 2 scrapie sheep, 2 normal sheep, and 2 scrapie goats.

3. *Boyden-Andersen Test.* The technique was as described by Boyden and Andersen (1955). No difference was observed between sera from 2 scrapie sheep and 2 normal sheep.

4. *Passive Anaphylaxis (Systemic).* Guinea-pigs were inoculated intraperitoneally with 2 c.c. of scrapie serum, and, the next day, inoculated intracardially with 0.5 c.c. of scrapie cerebrospinal fluid. Four cerebrospinal fluids were tested against 5 scrapie goat sera. No systemic shock was observed.

5. *Passive Anaphylaxis (Skin).* Guinea-pigs were inoculated intradermally with scrapie serum and, the next day, inoculated intradermally in the same site with scrapie cerebrospinal fluid. Four antigens and 5 sera were used; no skin reaction was observed.

6. *Complement-fixation Test.* Spleen antigen from a scrapie sheep at an early clinical stage of infection was used. Five scrapie sheep and 5 normal sheep sera were tested against several dilutions of the antigen, and no difference was noted between the two types of sera.

7. *Conglutinin Titres.* Conglutinin titres were obtained by a method similar to that described by Marks and Coombs (1957). No difference was observed between 6 scrapie sheep and 6 normal sheep, nor between 4 scrapie and 4 normal goats.

8. *Wassermann Test.* Five scrapie sheep sera, after inactivation at 62.5° C., all gave negative reactions and after inactivation at 56° C. one serum gave a doubtful reaction. Five further scrapie sheep sera and 5 normal sheep sera, after inactivation at 56° C., all gave negative reactions; some scrapie and some normal sera were anti-complementary. It was concluded that there were no significant differences between the scrapie and normal sera tested.

9. *Kahn Test.* This was conducted on 5 scrapie sheep sera and 5 normal sheep sera, which had been inactivated at 56° C. All the sera gave positive reactions, which were presumed non-specific.

10. *Haemagglutination Test.* Tests were conducted to observe if fluids, assumed to contain the scrapie agent, caused haemagglutination of erythrocytes from various species—in the same manner as, *e.g.* influenza virus material agglutinates avian erythrocytes; this would have enabled one to search for antibody using inhibition tests. One drop (0.05 c.c.) of a 2.5 per cent. suspension of erythrocytes was added to 0.5 c.c. amounts of undiluted cerebrospinal fluid derived from 2 scrapie goats. Erythrocytes from mouse, guinea-pig, hamster, rabbit, rat, sheep, goat, and fowl were used. No haemagglutination was observed after 2 hours' incubation at bench temperature.

The Wassermann and Kahn tests were conducted at the Royal Berkshire Hospital by courtesy of Dr. Hampson and Mr. Elcoate. I also wish to thank

Dr. W. S. Gordon, C.B.E., for his interest and encouragement.

Yours faithfully,
R. L. CHANDLER.

Agricultural Research Council,
Field Station,
Compton,
Nr. Newbury,
Berks.

January 7th, 1959.

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GENERAL

Tissue Vaccines in the Treatment of Bovine Papillomas

Sir,—We read with great interest the article in THE VETERINARY RECORD of November 29th, 1958, on the treatment of bovine papillomata.

With regard to the aetiology of the condition we feel that differentiation should be made between true papillomata, *i.e.* benign epithelial tumours, in this case of stratified squamous epithelium, and warty outgrowths probably of viral origin. It is the latter with which the article is really concerned.

The morphological differences seen in bovine warts would seem to indicate more than one causal agent. Two varieties are most frequently encountered: (a) the simple, usually small, finger-like outgrowths to be found, often in large numbers, around the teats and udders of heifers; and (b) the larger, more complex, verrucose angleberries commonly seen on the neck and abdomen of bullocks. Histologically there is a considerable difference in the proportion of fibrous tissue found in these two types.

It would appear that there is a distinct age incidence—animals affected are usually 1 to 3 years old. On some farms warts appear on the bulling heifers annually. On such farms the same structural type of wart is seen year after year and on at least one occasion an excellent response has been obtained with tissue vaccine prepared from the previous year's lesions. This suggests that prophylaxis might be a practical proposition.

Whilst angleberries are the more obvious and repulsive lesions they rarely affect more than a few individuals in a group. The teat warts, in our experience, are usually a herd problem and, if badly affected heifers are allowed to calve down without treatment, it may be impossible to milk them by hand or machine thus resulting in economic loss to the owner.

Papillomata, angleberries, or warts are probably still subjected to a greater variety of treatments than any other non-metabolic condition encountered in agricultural practice. Ringworm is possibly their only serious rival and with the advent of griseofulvin it may well be that it will become a bad second.

Consider the whole gamut of remedies. It starts with frank sorcery as exemplified by the "halfpenny purchase." One may scoff at old wives' tales and medieval witchcraft but even to-day one hears of such cures. Who knows but that this delightfully whimsical practice was, in effect, the fore-runner of twentieth-century psycho-analysis, and that the humble wart will, in due course, be considered of psycho-somatic origin?

The local application of infusions of milk-weed, ragwort, salicylic acid, friar's balsam, castor oil, and white arsenic all have their champions. Injections of colchicine, iodine, iron, antimony, and vitamin A are all tried with varying degrees of success.

So legion are the treatments that one suspects most are exhibited with pious hope but without inner conviction. Even in this age of broad-spectrum antibiotics and cortico-steroids we should hesitate to discount *vis medicatrix naturae*. As the authors rightly point out, in dealing with complaints like warts spontaneous recovery probably occurs with considerable frequency. When a single individual is cured one may publicly accept the credit, but experience should make one sceptical in the absence of controls.

Tissue-vaccines, both autogenous and non-autogenous, have formed the basis of "papilloma" treatment in this practice for the past 10 years. The vaccines we use were, it is believed, originally suggested and prepared by Dr. G. O. Davies of Liverpool for the late H. W. Steele-Bodger. We find autogenous vaccines tend to give better results than non-autogenous. The Ulster and Davies vaccines seem to differ in preparation—the latter being prepared in crystal violet. When collecting material for vaccine preparation it is wise where practicable to follow certain criteria:—

1. Specimens of all types of wart should be collected.
2. They should be taken from different parts of the body.
3. Warts should be chosen from as many affected members of the group as possible.

The line of treatment described in the article implies individual preparation of vaccine. This is much more applicable in the field to individual rather than herd problems—it is in the latter case that we consider the Davies method more appropriate. Our usual course of treatment consists of two subcutaneous injections of 1 to 2 c.c. vaccine at an interval of 2 to 3 weeks. A few years ago we injected 2 groups of Friesian heifers intradermally with 0.1 c.c. vaccine using a dental syringe. The owner of the stock was quite satisfied with the vaccine's effectiveness but the results obtained were in fact slower and less uniform. Since the vaccine was not expensive there seemed little point in decimating the dose and the method has not been repeated.

The first noticeable result of vaccination appears

to be stimulation of the wart to a mushroom-like rate of growth. So marked is the effect that some owners have become alarmed; the mode of action seems to be similar to that experienced with hormonal weed-killers. We would agree with the Ulster workers that there is a reduction in blood supply with consequent increase in friability. The neck of the wart, if present, becomes constricted and the tendrils fan out. This has the beneficial effect, in large angleberries, of exposing the deep fissures to the air, leading to reduction of the purulent secondary infections, lessening of the foetid smell and general desiccation. After the warts have dropped off no scar is left as is the case following simple ligation.

We should be interested to know whether the authors have tried injections of lithium antimony thiomalate in cases where tissue vaccines have not completely eradicated warts. Multiple buccal warts in puppies usually respond to treatment with haematinics but obstinate cases do occur—have tissue vaccines been tried in this condition? So-called "bleeding-warts" are found in the region of the withers and coxal tubers of cattle—have the authors any suggestions as to their aetiology or treatment?

In conclusion we should like to congratulate the authors on their excellent article which clearly shows that there is at least one rational scientific treatment for this interesting condition.

Yours faithfully,
A. STEELE-BODGER.
D. WRIGHT.

10, Bore Street.
Lichfield,
Staffs.

January 5th, 1959.

Slaughter of Animals (Prevention of Cruelty) Regulations, 1958

Sir,—I see that these regulations still contain the rule that no animal may be slaughtered in sight of another. Enforcement of this regulation never prevents any suffering and very frequently gives rise to considerable fear and even terror.

The rule is based, I believe, on the findings of a Royal Commission about 40 years ago, to the effect that "in the absence of evidence to the contrary" it must be assumed that animals have an instinctive knowledge and fear of death. The evidence to the contrary which was *not* absent then or now is that cattle, sheep, and swine continue to graze in close vicinity of bodies of their comrades dead from disease, accident, or other causes, and swine will frequently eat such bodies.

What frightens animals of all species is pain or the sight, sound, or smell of things that experience has taught them may cause pain, *i.e.* men that flourish sticks and shout at them, noisy machinery, and the like. With the gregarious animals there must also be included anything that breaks the "protected" feeling that the presence of their comrades inspires.

In many places, in order to fulfil the requirements of this regulation, the animals must be roped one at a time and dragged round a corner. The roped animal struggles furiously and often groans loudly; surely evidence of extreme terror at being dragged away from the herd. The animals remaining in the pen struggle hard to follow him and can only be prevented by quick action by the men at the gate, often with the use of sticks and shouts. The animals will mill about for several minutes after the gate is closed which is surely evidence of fear. In fact, from the moment work starts, none of the animals is free from fear. The last two or three in the pen are often nearly uncontrollable with terror.

In properly constructed abattoirs there are lanes leading from the pens to the slaughter hall, narrowed at the end so that the animals are in single file. There is rarely need for much compulsion in getting the first animal along, and the rest follow with no compulsion at all and no signs of fear. The final roping when it is necessary only frightens the animal being roped. The remainder, since they cannot see what is happening, are not alarmed.

Some 10 years ago, I wrote to you indicating the use that can be made of decoy animals; animals kept at the abattoir and accustomed to walk along the lanes and to stand in the slaughter halls and pens and any place where loneliness might give rise to fear. The full use of such animals would be illegal under the present regulations since they would at times be in sight of animals being slaughtered.

Considerable attention has been given to the reduction and, when possible, the elimination of the pain, admittedly slight, and terror, admittedly of very short duration, associated with the final opening of the arteries; but little has been done to prevent or reduce the pre-slaughter fear which can be both deep and long. I have indicated two lines which future legislation should make compulsory, both based on available evidence—the observed behaviour of animals. Legislation based on "absence of evidence to the contrary" should be abolished.

The slaughter of horses involves some different problems as their mentality differs in some respects from that of the animals mentioned above. That of dogs and cats involves a totally different approach. If any of your readers care to pursue the subject, I hope they will not confuse the issue by introducing discussions on the mentality of these animals, as some did when I first wrote on this subject.

Yours faithfully,
RODERICK MACGREGOR.

Kildrummie,
Betchworth,
Surrey.

January 12th, 1959.

ADVERTISERS' ANNOUNCEMENTS

THE DISTILLERS COMPANY (BIOCHEMICALS) LIMITED announces the introduction of "Mixtamycin" Solution Veterinary. Each vial contains 5.0 grammes strentomycin sulphate and 5.0 grammes dihydrostreptomycin sulphate in 30 ml. solution ready for injection.

PARKE, DAVIS & COMPANY announce that, with effect from January 19th, 1959, a new Parke, Davis veterinary product, Chloromycetin Vetrettes, 500 mg., will be available for oral and intrauterine administration to large and small animals.